

Correlation of discocyte frequency and ATP concentration in preserved blood. A morphological indicator of red blood cell viability

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Abstract

Red blood cells (RBC) are viable if kept in an adequate preservative solution, although gradual changes in morphology and metabolism may occur. There is a gradual decrease in adenosine-5'-triphosphate (ATP) concentration, pH, glucose consumption, and enzyme activity during preservation. The normal discocyte shapes are initially replaced by echinocytes and stomatocytes and, at final stages, by spherocytes, the last step before splenic sequestration. Post-transfusional survival has been correlated with the ATP concentration. RBC preserved in ADSOL, a solution containing adenine, dextrose, sodium chloride, and mannitol, are viable for transfusion for up to 6 weeks. Erythrocytes from 10 blood units taken from healthy adult donors were preserved for 12 weeks in ADSOL at 4°C. We now report a significant correlation ($r^2 = 0.98$) between the percentage of discocytes (89 to 7%) and ATP (100 to 10%) concentration in ADSOL-preserved RBC. The results suggest that the percent of discocyte shapes used as an indicator of ATP concentration may be a useful indicator for quality control of RBC viability in centers which have limited assay facilities.

Key words

- Red blood cells
- Adenosine-5'-triphosphate
- Blood transfusion

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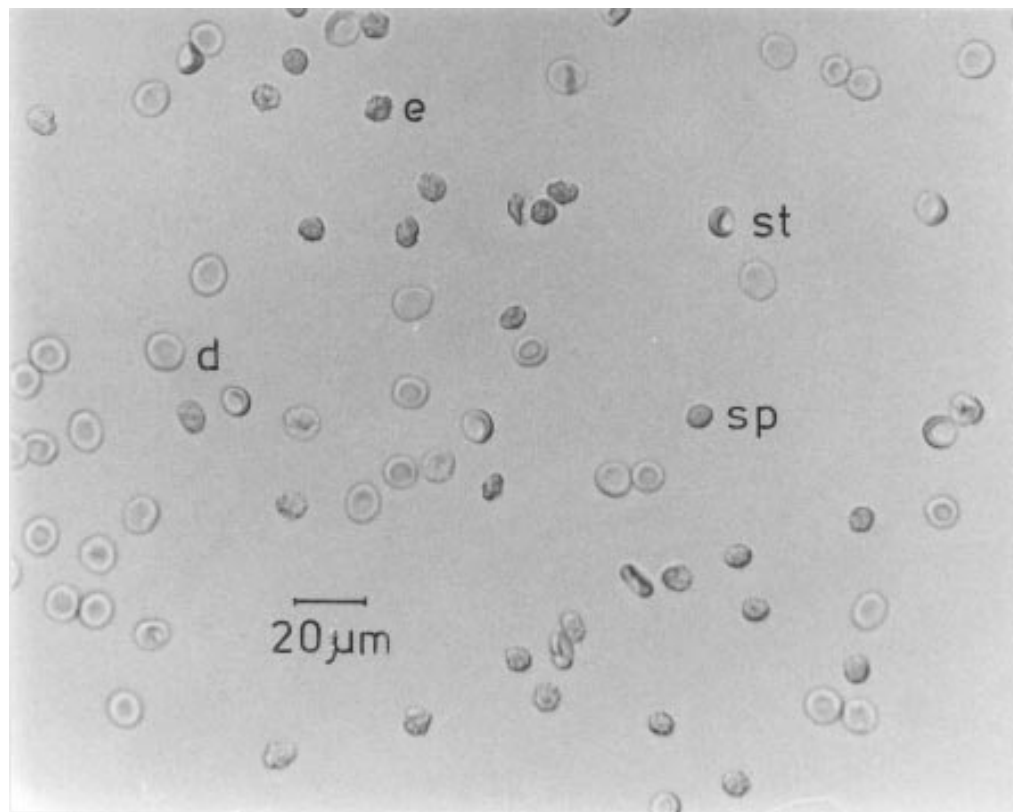
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ADSOL, a preservative solution containing adenine, dextrose, sodium chloride, and mannitol, is employed worldwide. Erythrocytes maintained *in vitro* in this medium (AS-1) are viable for transfusion up to 6-7 weeks (1). Erythrocytes maintained *in vitro* under different conditions generate energy via anaerobic glycolysis which is followed by organic acid accumulation, pH decrease, and enzyme decay (2-5). Post-transfusional survival has been correlated with adenosine-

5'-triphosphate (ATP) concentrations and morphological changes of transfused red blood cells (RBC) (6,7). Under different conditions, it has been shown that RBC with a high ATP concentration keep the discocyte shape and, as ATP decreases, RBC change into echinocytes and finally into spherocytes (8).

Venous blood units were collected in an experimental quadruple blood-pack with a primary container having 63 ml of CPDA-1

Figure 1 - Photomicrograph of human erythrocytes preserved in ADSOL for 5 weeks at 4°C. d, Discocyte; e, echinocyte; st, stomatocyte; sp, spherocyte.



(preservative solution with citrate, phosphate, dextrose, and adenine), and 3 satellite packs, one containing 100 ml of ADSOL and the others being empty. Blood samples (450 ml) were taken from 10 healthy adult donors and centrifuged at 600 *g* at 4°C, and the erythrocytes from the primary container were resuspended in the same volume of ADSOL. The suspensions were transferred to sterile plastic vials and kept at 4°C. All assays were performed on the day following collection, and at intervals up to 12 weeks. Erythrocyte suspensions were diluted to 1:200 in ADSOL and classified in a Neubauer-improved hemocytometer using light microscopy within 10 min, according to the morphological criteria of Bessis (9), simplified by Leonart (10). Discocytes, echinocytes, stomatocytes and spherocytes were counted in samples containing 1000 cells, as illustrated in Figure 1.

ATP concentrations were assayed by the

method of Beutler (11) for 12 weeks. The mean of the initial values for 10 independent measurements ($4.5 \pm 0.3 \mu\text{mol ATP/g hemoglobin}$, equivalent to 100%) decreased gradually and was correlated with the percentage of discocytes. Gradual changes from discocytes to spherocytes were observed over the 12 weeks of preservation. Morphological indices have been considered to be important items for quality control of RBC during storage for hemotherapy (12). Echinocytes appeared at the beginning of storage and increased up to the 6th week, when they were gradually replaced by spherocytes. The results obtained during the first 6 weeks of preservation are consistent with those observed in ADSOL (13) and SAGM (saline, adenine, glucose, and mannitol) (14). RBC preserved in ADSOL (13), SAGM (14) or in the presence of DEHP (di-(2-ethylhexyl) phthalate) (12) showed better morphological indices when compared with those stored

in CPD (citrate, phosphate, and dextrose) or CPDA-1. Changes in ATP concentrations and hemolysis of RBC do occur during RBC preservation, and are related to the activity of membrane ATPase and therefore indicate functional integrity of the membrane (15).

Our data show that the decrease in the percentage of discocytes is positively correlated with the decrease in ATP concentration ($r^2 = 0.98$) during storage in ADSOL (Figure 2).

The analysis of the percentage of discocyte shapes providing an index for ATP concentration could be a useful tool in blood bank quality control since it may indicate RBC viability.

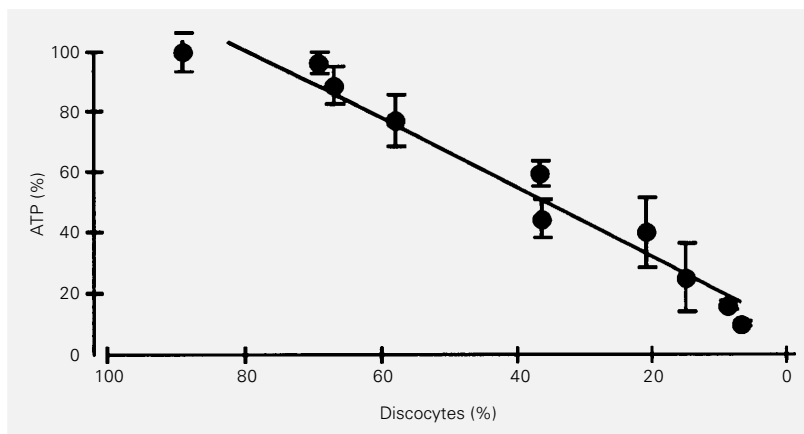


Figure 2 - Correlation between ATP concentration and percent of discocytes in blood preserved in ADSOL for 12 weeks. Data are reported as means \pm SD for 10 samples. One hundred percent ATP corresponds to 4.5 ± 0.3 μ mol ATP/g hemoglobin. The circles represent the experimental data, and the full line was calculated using the best fit method.

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